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Bone marrow transplantation attenuates murine IgA nephropathy: Role of a stem cell disorder[1]

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Bone marrow transplantation attenuates murine IgA nephropathy: Role of a stem cell disorder.

Background. The pathogenesis of IgA nephropathy is still obscure. The aim of this study was to investigate whether the fundamental pathogenesis of IgA nephropathy lies in bone marrow stem cells (BMCs).

Methods. We used donors of two different strains for bone marrow transplantation (BMT) into mice with a high content of serum IgA (ddY strain, HIGA mice), a murine model of IgA nephropathy. One group (B6→HIGA, $N = 5$) received BMCs of C57BL/6j (B6) mice, and the other (HIGA→HIGA, $N = 8$) were reconstituted with BMCs of HIGA mice.

Results. Twenty-six weeks after BMT, in B6→HIGA mice, mesangial deposits of IgA and C3 were statistically milder than those in HIGA→HIGA mice. Light microscopic observations disclosed that glomerular sclerosis and mesangial matrix expansion in B6→HIGA mice were decreased compared with those in HIGA→HIGA mice. These B6→HIGA mice also excreted less urinary albumin than HIGA→HIGA mice. Furthermore, serum levels of IgA in B6→HIGA mice were markedly lower than those in HIGA→HIGA mice. Size analysis of serum IgA revealed that macromolecular IgA were notably lower in B6→HIGA mice than in HIGA→HIGA mice.

Conclusions. Our results suggest that qualitative and quantitative changes of serum IgA are determined at the level of stem cells, and that BMT from normal donors can attenuate glomerular lesions in HIGA mice. This approach may offer a new avenue to study the pathogenesis of IgA nephropathy.

Immunoglobulin A (IgA) nephropathy is the most frequently presented form of glomerulonephritis, and is defined by the deposition of IgA in glomerular mesangium [1, 2]. IgA nephropathy was initially considered to be a benign disease until it was recognized that a consistent percentage of the patients, despite standard therapy, had a progressive course leading to end-stage renal disease (ESRD) requiring dialysis [3–5]. Although the pathogenesis of IgA nephropathy is still obscure, some immunological dysfunction seems to play an important role [5–9].

In some patients with IgA nephropathy who presented with ESRD and subsequently were transplanted with healthy kidneys, IgA nephropathy recurred in the grafts [10]. Additionally, when a kidney with mesangial IgA deposits was inadvertently transplanted into a patient whose ESRD was unrelated to IgA nephropathy, the deposits disappeared [11]. These examples indicate that an intrinsic circulating factor(s), rather than an intrinsic defect(s) of the kidney, caused the IgA deposition in the mesangial area.

Recently, we encountered a patient with IgA nephropathy and chronic myeloblastic leukemia whose bone marrow transplantation (BMT) not only cured the leukemia, but also eliminated the mesangial IgA deposits (abstract; Sakai, *Nephrology* 3:2–3, 1997). This exciting observation provided the first evidence suggesting that abnormalities of bone marrow stem cells (BMCs) may be involved in the pathogenesis of IgA nephropathy and that BMT from a normal donor might have attenuated glomerular lesions in our patient. However, some possibility remains that the immunosuppressant used for our patient may have effected the renal recovery.

In the present study, we employed a murine model of IgA nephropathy, high serum IgA ddY (HIGA) mouse, whose mesangial IgA deposition spontaneously became apparent by approximately 25 weeks of age [12]. The mesangial IgA deposition became more marked with age, and expansion of the mesangial area with electron-dense

[1] **See Editorial, p. 1964.**

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deposits was also observed [12]. In sera of HIGA mice, macromolecular IgA was increased [12]. Similarly, an increase of serum IgA, especially macromolecular IgA, was observed in patients with IgA nephropathy [13]. Macromolecular IgA was considered to be one candidate of circulating factors to have a pivotal role in the pathogenesis of human IgA nephropathy [6, 9]. Therefore, the HIGA mouse is an appropriate model in which to study the pathogenesis of IgA nephropathy.

To investigate whether the pathogenic agent of IgA nephropathy lies in BMCs and, accordingly, whether BMT might be a tool for treating IgA nephropathy, HIGA mice (23 to 25 weeks of age) received BMCs from donors of two strains: normal C57BL/6j (B6) mice (25-week-old) and syngeneic HIGA mice (27-week-old). The results suggest that serum levels of IgA, mainly macromolecular IgA, may be determined by a factor(s) in BMCs that can cause IgA nephropathy and, consequently, that BMT have a beneficial effect on murine IgA nephropathy.

METHODS

Mice

Female HIGA mice were obtained from Nippon Shinyaku Co. Ltd. (Kyoto, Japan). Female B6 mice were purchased from Japan Sankyo Labo Service Corporation, Inc. (Tokyo, Japan). All of the animals were maintained on autoclaved standard laboratory chow under specific pathogen-free conditions in the animal facility at Tokyo Metropolitan Institute of Gerontology. All surgical procedures conformed to the guidelines established by the U.S. Department of Health and Human Services, as published by the National Institutes of Health (NIH publication no. 85-23, revised 1985).

Experimental protocol

One week before BMT (pre-BMT), serum and urine were obtained from recipient HIGA mice (22 to 24 weeks of age), and then, after anesthesia with pentobarbital, approximately 2 mm cubes of renal sections were removed surgically. On the day of cell transfusion, recipients were lethally irradiated (8.5 Gy/mouse) and then divided into two groups: One group (designated as B6→HIGA, $N = 5$) received BMCs of 25-week-old B6 mice, and the other (designated as HIGA→HIGA, $N = 8$) were reconstituted with BMCs of 27-week-old HIGA mice. Five to six hours after irradiation, recipient HIGA mice (23 to 25 weeks of age) were injected intravenously with respective 1×10^7 T-cell–depleted BMCs. From six weeks after transplantation, we obtained serum samples every four weeks. Twenty-six weeks after the reconstitution (approximately 50-week-old, post-BMT), all mice were sacrificed to obtain serum, urine, kidney specimens, and spleen cells. Serum and urine samples for immunological assay were kept at −80°C until use. Kidney specimens for immunohistopathology were embedded in OCT compound (Miles Scientific, Napperville, IL, USA) and quickly frozen in dry ice and acetone. Spleen cells were subjected to immunocytological analysis on the day of sacrifice.

Irradiation and T-cell–depleted bone marrow cells

Preliminary experiments showed that a single 8.5 Gy dose of total body irradiation from a ^{60}Co source killed all HIGA mice by three weeks after the irradiation if subsequent BMT was not carried out. In spleens of these irradiated mice, no hematopoietic colonies were visible macroscopically 14 days after irradiation, indicating that no pluripotential stem cells were present [14]. Conversely, autologous BMT rescued all HIGA mice exposed to 8.5 Gy without side effects such as gastrointestinal bleeding or infection. Based on these results, all recipient HIGA mice were irradiated with 8.5 Gy.

Bone marrow cells from pelvis, femoral, and peroneal bones were incubated with anti–Thy-1.2 Ab (F7D5; Serotec Ltd., Oxford, UK) at a 1:1000 dilution on ice for 30 minutes and then reacted with rabbit complement (Cedar Lane, Ontario, Canada) at 37°C for 30 minutes. Preliminary studies confirmed that this procedure eliminated more than 98% of the T cells, as revealed by immunofluorescent staining.

FACS analysis

Spleen cells were gently homogenized and depleted of red blood cells by Tris-buffered ammonium chloride (pH 7.2). To prevent nonspecific binding of Abs to Fcγ receptors, spleen cells were incubated with purified mouse IgG (Zymed Laboratories, Inc., San Francisco, CA, USA) on ice for 30 minutes. After washing, phycoerythrin-conjugated monoclonal mouse anti-H-2D^b Ab (Pharmingen, San Diego, CA, USA) was reacted with the pretreated cells on ice for 30 minutes. Then cells were resuspended in 1% paraformaldehyde containing 0.1% azide. Stained cells were analyzed by FACScan (EPICS CS; Coulter Electronics, Hialeah, FL, USA).

Histopathological analysis

For light microscopy, tissues embedded in paraffin were stained with periodic acid-Schiff (PAS) reagent. For assessment of histological changes, the frequency of globally or segmentally sclerosed glomeruli was calculated in each section by dividing the number of affected glomeruli by the total number of glomeruli. The extent in mesangial matrix increase was assessed for each glomerulus by scoring from 0 to 3: 0 = normal, 1 = mild (<one third of glomerular tuft area), 2 = moderate (<two thirds of glomerular tuft area), and 3 = severe (>two thirds of glomerular tuft area). The glomeruli were scored in the average of 56 glomeruli (range, 22 to 102 glomeruli) cross sections per specimen, and a value

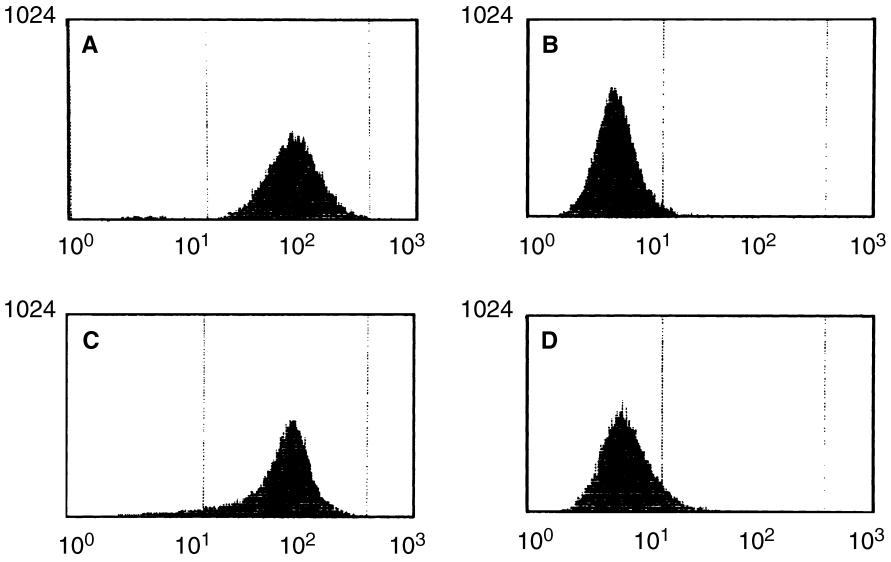

Fig. 1. FACS analysis of lymphocytes in spleens 26 weeks after bone marrow transplantation (BMT). Splenic lymphocytes depleted of red blood cells were stained with anti–H-2D^b antibodies (Ab) and were then subjected to FACS. Results represent (*A*) 50-week-old B6 mouse, (*B*) 50-week-old HIGA mouse, (*C*) B6→HIGA mouse 26 weeks after BMT, and (*D*) HIGA→HIGA mouse 26 weeks after BMT.

for each individual animal represents an averaged score in each section.

Cryostat sections were cut 4 μm thick and fixed in cold acetone for 10 minutes. For direct immunofluorescence, the sections were stained with fluorescein isothiocyanate (FITC)-labeled goat anti-mouse IgA, IgG, IgM, and complement C3 Abs (Cappel Research Products, Durham, NC, USA) appropriately diluted in phosphate-buffered saline (PBS) at 37°C for one hour. Immunohistopathological analysis was carried out under a Zeiss Axiophot fluorescence photomicroscope. The relative amount of staining was scored semiquantitatively as follows [12]: 0 = negative staining, 1 = slight (focal and weak) staining, 2 = moderate staining, and 3 = marked (diffuse and intense) staining. More than 20 glomeruli per specimen were scored in this manner, and the mean was calculated by the total score of affected glomeruli by the total number of glomeruli as fluorescent intensity (FI) in each individual animal.

Urinary albumin concentration

Concentrations of excreted urinary albumin were determined by the single radial immunodiffusion method (SRID) [15]. In brief, urine samples and a mouse albumin reference (Cappel Research Products) as a standard were applied to wells on agarose gel-containing polyclonal rabbit anti-mouse albumin Ab (Cappel Research Products). By this method, we could minimally detect 60 μg/ml of mouse albumin. Because many samples were in an undetectable range, excreted urinary albumin concentrations measured by SRID were grouped for the statistical analysis as 0, 0 to 99 μg/ml; 1, 100 to 199 μg/ml; 2, 200 to 299 μg/ml; and 3, 300 to 399 μg/ml.

Enzyme-linked immunosorbent assay

Concentrations of mouse immunoglobulins were measured by enzyme-linked immunosorbent assay (ELISA) as follows. Ninety-six-well plates (IMMULON 2; Dynex Technologies, Inc., Chantilly, VA, USA) had been previously coated with goat affinity-purified antimouse immunoglobulin Abs (Cappel Research Products). After reacting with PBS containing 1% skim milk powder to block nonspecific reactivity of sera, the plates were incubated with appropriately diluted samples and immunoglobulin reference sera (ICN Pharmaceuticals Inc., Costa Mesa, CA, USA) and then washed with PBS containing 0.05% Tween20 (PBS/Tween). Adsorbed immunoglobulin was incubated with peroxidase-labeled goat antimouse immunoglobulin Ab (Zymed Laboratories). After washing with PBS/Tween, the residual peroxidase was reacted with TMB peroxidase substrate (Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD, USA) according to manufacturer's instructions. Absorbance in each well was measured with a MTP-100 microplate reader (Corona Electric Co., Tokyo, Japan) at a wavelength of 450 nm.

Fast protein, peptide, and polynucleotide liquid chromatography

Ten-times diluted serum samples pooled from mice of each group were subjected to the fast protein, peptide, and polynucleotide liquid chromatography (FPLC) system (Pharmacia, Uppsala, Sweden) on a Superdex 200 HR 10/30 (Pharmacia). Molecular weight markers were thyroglobulin (669 kDa) and aldolase (158 kDa; Pharmacia). Samples were applied to the FPLC column at a flow rate of 0.4 ml/min, and fractionated samples were collected every one minute in 0.1 M phosphate buffer, pH 7.0, containing 0.1 M NaCl.

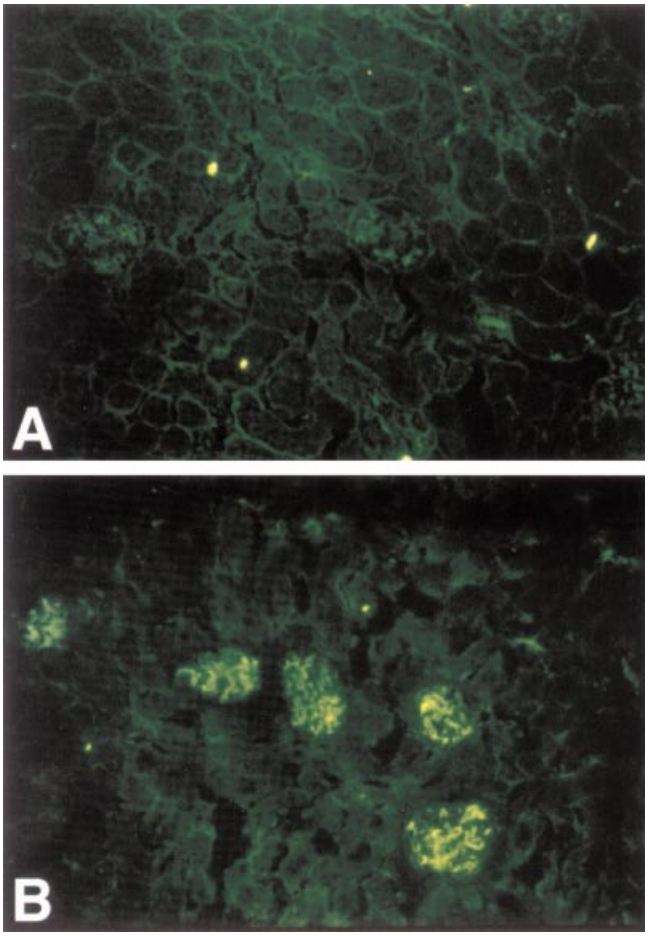

Fig. 2. Representative immunofluorescence photographs of IgA staining in renal tissues (original magnification ×200) of (*A*) B6→HIGA mouse at post-BMT and (*B*) HIGA→HIGA mouse at post-BMT. Frozen sections were stained directly with FITC-labeled antimouse IgA Ab. At post-BMT, glomeruli in B6→HIGA mice showed weak IgA deposition. In contrast, HIGA→HIGA mice at post-BMT had global, diffuse mesangial IgA deposits.

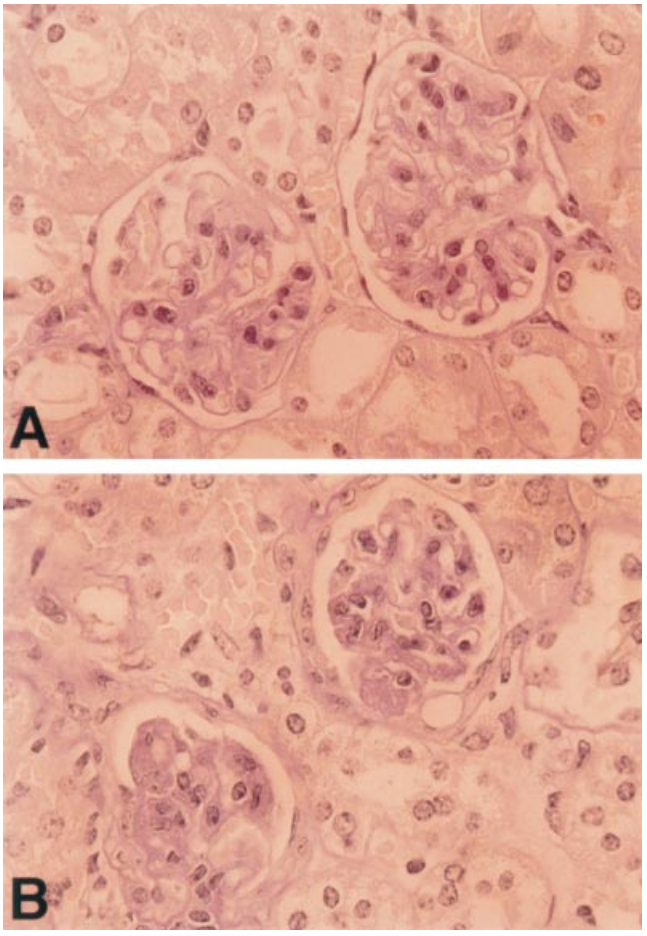

Fig. 4. Representative microscopic photographs in renal tissues at post-BMT (PAS staining, original magnification ×400). (*A*) Glomeruli in B6→HIGA mouse showed a normal appearance. (*B*) In contrast, glomeruli in the HIGA→HIGA mouse had severe mesangial matrix expansion and segmental glomerulosclerosis.

Statistical analysis

All values are expressed as means ± SEM. The two-group paired *t*-test was used to compare the changes of histology at pre- and post-BMT in each individual animal; the unpaired *t*-test was used to analyze ELISA and histology data between transplanted animals, and the Mann–Whitney test was used to compare urinary data. A P value of less than 0.05 was considered statistically significant.

RESULTS

Donor bone marrow stem cells repopulated in recipients

To confirm that BMCs from each donor successfully repopulated in recipients, H-2 typing was performed 26 weeks after BMT. Splenic lymphocytes were stained with anti–H-2D^b Ab and then analyzed by FACS. Lymphocytes of nontransplanted B6 mice, whose MHC class I was H-2D^b, showed positive staining (Fig. 1A), whereas lymphocytes in HIGA mice, whose MHC type has not yet been determined, were negative for H-2D^b (Fig. 1B). This method enabled us to recognize whether lymphocytes in recipients were B6 or HIGA in origin. Accordingly, lymphocytes in HIGA mice received BMCs of B6 mice (B6→HIGA) were positive like those of B6 mice (Fig. 1C). HIGA recipients of BMCs of HIGA mice (HIGA→HIGA) had H-2D^b-negative lymphocytes (Fig. 1D). Thus, BMCs transferred to B6→HIGA mice successfully replaced those of the HIGA type.

Immunodeposition decreased in glomeruli of B6→HIGA mice

Because BMCs of B6→HIGA mice originated from B6 mice, we next examined mesangial depositions of

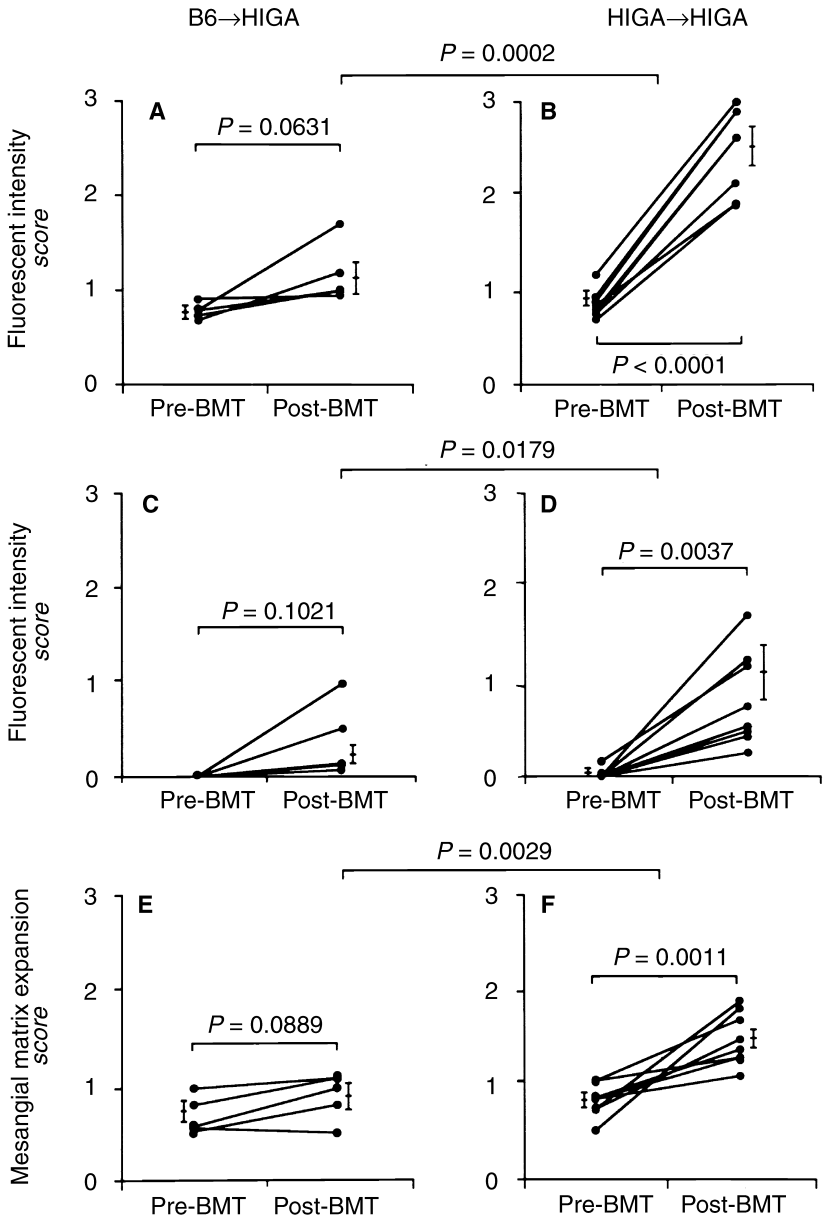

Fig. 3. Comparison of mesangial immunofluorescent intensity and mesangial matrix expansion scores before and after BMT. (*A*) IgA staining in B6→HIGA mice ($N = 5$). (*B*) IgA staining in HIGA→HIGA mice ($N = 8$). (*C*) C3 staining in B6→HIGA mice. (*D*) C3 staining in HIGA→HIGA mice. (*E*) Mesangial matrix expansion scores of B6→HIGA mice. (*F*) Mesangial matrix expansion scores of HIGA→HIGA mice. Bars are means ± SEM. Renal sections biopsied before and 26 weeks after BMT were stained for IgA and C3, followed by scoring more than 20 glomeruli per section and calculating the values of FI as described in the **Methods** section. The degree of mesangial matrix expansion was semiquantitatively calculated as detailed there. *P* values are indicated between pre- and post-BMT in each group (paired *t*-test) or between B6→HIGA mice at post-BMT and HIGA→HIGA mice at post-BMT (unpaired *t*-test).

immunoglobulins and C3 by immunofluorescent staining. At the post-BMT period (26 weeks after BMT; when mice were approximately 50 weeks old), mesangial deposition of IgA was weak in B6→HIGA mice (Fig. 2A). At the same time, IgA deposits in HIGA→HIGA mice were observed diffusely and globally in the mesangial area (Fig. 2B).

When we then scored the intensity of fluorescence (FI), mesangial IgA depositions in both B6→HIGA ($N = 5$) and HIGA→HIGA ($N = 8$) mice were similarly slight at pre-BMT (one week before BMT; Fig. 3 A, B). In B6→HIGA mice, the FI values of IgA depositions at post-BMT were not statistically different from those at pre-BMT (1.17 ± 0.14 vs. 0.79 ± 0.03, $P > 0.05$; Fig. 3A). The degree of mesangial IgA depositions in B6→HIGA mice at post-BMT was as same as those in nontransplanted normal B6 mice at the age of 50 weeks (1.34 ± 0.32). In sharp contrast, all HIGA→HIGA mice had much higher FI scores for mesangial IgA deposition at post-BMT than those at pre-BMT (2.49 ± 0.16 vs. 0.84 ± 0.05, $P < 0.0001$; Fig. 3B). It should be noted that FI scores of mesangial IgA deposition in nontransplanted HIGA mice at 50 weeks old were comparable to those of post-BMT HIGA→HIGA mice (2.39 ± 0.24). At post-BMT, mesangial IgA depositions in B6→HIGA mice were statistically milder than those in HIGA→HIGA mice ($P < 0.01$) or age-matched HIGA mice. Clearly, mesangial IgA depositions of B6→HIGA mice took a

turn for the better compared with those of HIGA→HIGA mice or with nontransplanted HIGA mice.

Next, mesangial complement C3 deposition was analyzed because such deposits universally accompany human and murine IgA nephropathy [5, 12]. At pre-BMT, mesangial depositions of C3 were seldom apparent in either group of mice. In B6→HIGA mice, FI scores of C3 at post-BMT were comparable to those at pre-BMT (0.17 ± 0.08 vs. 0.0 ± 0.0, $P > 0.05$; Fig. 3C). However, in HIGA→HIGA mice, scores of C3 deposits were considerably higher after than before BMT (1.08 ± 0.25 vs. 0.02 ± 0.02, $P < 0.01$; Fig. 3D). FI scores of mesangial C3 depositions in B6→HIGA mice were statistically lower than those in HIGA→HIGA mice ($P < 0.05$ at post-BMT).

At post-BMT, mesangial depositions of IgG in B6→HIGA mice were also milder than those in HIGA→HIGA mice without a statistically significant difference (1.28 ± 0.32 vs. 1.80 ± 0.21, $P > 0.05$), whereas mesangial IgM depositions in B6→HIGA mice were the same as those in HIGA→HIGA mice (1.38 ± 0.32 vs. 1.49 ± 0.27).

Glomerular sclerotic lesion and mesangial matrix expansion diminished in B6→HIGA mice

Because immunofluorescent staining revealed that BMT from normal B6 mice attenuated mesangial IgA and C3 deposition in HIGA mice, this effect was analyzed with light microscopy. In both groups, no glomeruli had sclerotic lesions at pre-BMT. At post-BMT period, few glomeruli in B6→HIGA mice had sclerotic lesions. In fact, the frequency of glomerulosclerotic lesions in B6→HIGA mice (Fig. 4A) was significantly lower than that in HIGA→HIGA mice (0.40 ± 0.40 vs. 9.8 ± 1.5%, $P < 0.001$; Fig. 4B). Next, we scored the degree of mesangial matrix expansion and calculated the values of matrix expansion scores as described in the **Methods** section. In B6→HIGA recipients of BMT, scores at post-BMT were not statistically higher than those at pre-BMT (0.90 ± 0.11 vs. 0.69 ± 0.10, $P > 0.05$; Fig. 3E). Conversely, the mesangial matrix expansion scores of HIGA→HIGA mice were considerably higher after than before BMT (1.49 ± 0.10 vs. 0.76 ± 0.06, $P < 0.01$; Fig. 3F). At post-BMT, the mesangial matrix expansion scores of B6→HIGA mice were statistically lower than those of HIGA→HIGA mice ($P < 0.01$). BMT from normal mice also improved the glomerular changes of HIGA mice, as judged by light microscopy.

Decreased concentration of excreted albuminuria from B6→HIGA mice

Next, we examined how these histological improvements effected urinary albumin excretion. Albumin excretion from HIGA mice was virtually undetectable at pre-BMT (<60 μg/ml; Fig. 5). However, at post-BMT, the scores of albuminuria in B6→HIGA mice were statistically lower than those from HIGA→HIGA mice ($P < 0.05$; Fig. 5). Therefore, in addition to ameliorating the renal lesions, BMT also reduced albuminuria, an important clinical feature of IgA nephropathy.

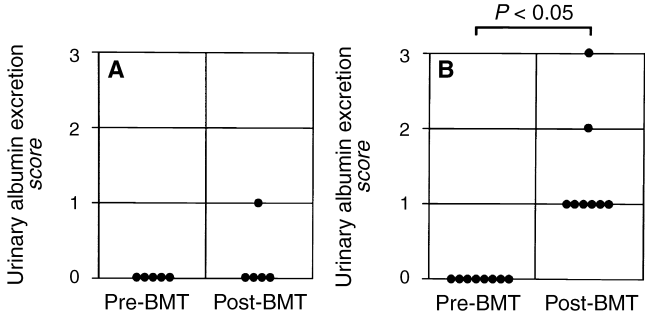

Fig. 5. Comparison of the concentration of urinary albumin. The concentration of albumin was measured by the single radial immunodiffusion method and was then scored for statistical analysis. This figure shows albuminuria in (*A*) B6→HIGA mice and in (*B*) HIGA→HIGA mice at pre- and post-BMT.

Serum IgA levels, which increase with age in HIGA mice, were decreased by bone marrow transplantation from B6 mice

To investigate the immunological components participating in these effects of BMT, we next calculated serum immunoglobulin levels by ELISA. Serum IgA levels (Fig. 6A) in B6→HIGA mice were much lower than those in HIGA→HIGA mice throughout the experimental course after BMT (402 ± 65 vs. 1519 ± 93 mg/dl, $P < 0.01$ at post-BMT). However, serum IgM levels (Fig. 6B) in B6→HIGA mice were significantly higher than in HIGA→HIGA mice from 14 weeks after BMT (85.0 ± 9.8 vs. 48.1 ± 2.2 mg/day, $P < 0.01$ at post-BMT). Serum IgG levels (Fig. 6C) in both groups changed similarly throughout the experimental course (1128 ± 79 vs. 1242 ± 74 mg/dl, $P > 0.05$ at post-BMT).

Bone marrow transplantation from B6 mice decreased serum macromolecular IgA of transplanted HIGA mice

To assess the size of serum IgA as a determinant of mesangial deposition at post-BMT, IgA level in samples fractionated by FPLC were measured by ELISA. Preliminary examination revealed that most of these IgA molecules in nontransplanted HIGA mice at 25 and 50 weeks of age were macromolecules (molecular weight >500 kDa). We also confirmed that macromolecular IgA was not predominant in serum of normal B6 mice at 25 and 50 weeks old. As in the HIGA mice, macromolecular IgA with a molecular weight of approximately 670 kDa predominated in HIGA→HIGA mice (Fig. 7). However, in B6→HIGA mice, levels of macromolecular IgA were

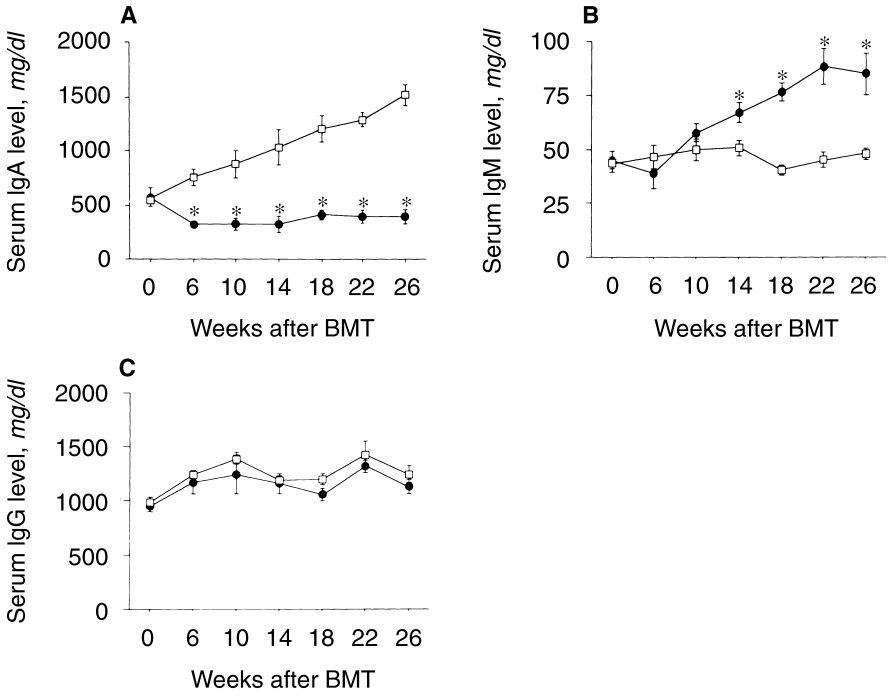

Fig. 6. Serum immunoglobulin levels determined by ELISA for (*A*) IgA, (*B*) IgM, and (*C*) IgG in B6→HIGA mice (●) and in HIGA→HIGA mice (□). Values are means ± SEM; $*P < 0.05$ vs. HIGA→HIGA mice. Serum samples were obtained before BMT and every four weeks after BMT.

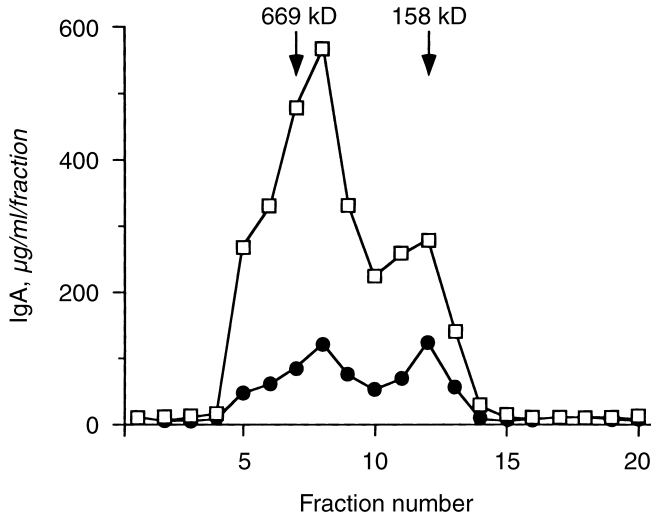

Fig. 7. Analysis of IgA size in sera from B6→HIGA mice (●) and HIGA→HIGA mice (□). Serum samples collected from mice of each group were fractionated by FPLC (0.4 ml/min, 1 ml/tube), and the concentrations of IgA in fractions were determined by ELISA. Monomeric IgA has been identified as about 160 kDa [24].

markedly lower than in HIGA→HIGA mice and were equivalent to those of monomeric IgA with a molecular weight of 160 kDa (Fig. 7).

DISCUSSION

When we compared IgA nephropathy-prone HIGA mice transplanted with BMCs from normal B6 mice (B6→HIGA) to their counterparts given HIGA BMCs to probe the origin of IgA nephropathy, our results showed clear-cut reductions in mesangial IgA and complement C3 deposition and a decrease in glomerular sclerotic lesions and mesangial matrix expansion only in the recipients of B6 cells. These B6→HIGA mice also excreted less urinary albumin than the HIGA→HIGA controls. Subsequent analysis of immunological changes revealed that the serum IgA level, especially macromolecular IgA, in B6→HIGA mice was much lower than in HIGA→HIGA mice.

For successful BMT and efficient observation of its effects, we needed a murine model in which mesangial IgA deposition occurred by the 25th week of life. The essential factors were that the thymus is considered crucial to immunological reconstitution after BMT [16], but thymic atrophy starts in mice at 25 weeks of age and worsens drastically thereafter [17]. Because HIGA mice, which originated from IgA nephropathy-prone ddY mice and produce especially high levels of serum IgA, exhibited mesangial IgA deposits at 25 weeks of age and these deposits become more marked with age [12], we chose them for this study.

In general, T-cell–depleted BMCs are the cells used in BMT [18, 19]. For the generation of naive T cells capable of differentiating into antigen-specific T lymphocytes through education in thymus, two to three months are required after BMT [20, 21]. In this study, 26 weeks after the transplantation of T-cell–depleted BMCs, donor BMCs were successfully regenerated in recipients, and the cell surface phenotype of lymphocytes in B6→HIGA mice completely changed from the HIGA type to the B6 type (Fig. 1). Additionally, the IgG level in

B6→HIGA mice was comparable, and the IgM content was superior to that in HIGA→HIGA mice (Fig. 6) or age-matched, unmanipulated HIGA mice (data not shown). These findings contradict some reports that immunodeficiency occasionally occurred in mice after lethal irradiation followed by transfer of multiple histocompatibility complex-mismatched BMT [22]. In our transplant recipients, no immunological defect was evident. Therefore, we considered it feasible to examine the role of BMCs in the pathogenesis of IgA nephropathy by analyzing mice 26 weeks after BMT.

As a result of BMT, mesangial IgA depositions in B6→HIGA mice were statistically weaker than those in HIGA→HIGA mice or age-matched HIGA mice (Figs. 2 and 3). Until now, mesangial IgA deposition has been attributed to a high production of IgA [23], the presence of serum macromolecular IgA [6, 13], the defective clearance of IgA [9], and abnormal cytokine production [8]. To pursue the study of factors that attenuate mesangial IgA deposition in B6→HIGA mice, we first analyzed the nature of serum IgA in this model.

As shown in Figure 6, serum IgA levels in B6→HIGA mice were much lower than in HIGA→HIGA mice. Others believe that not only in murine but also in human IgA nephropathy, mesangial IgA deposition correlates better with the amount of macromolecular IgA [13, 24, 25], because of the macromolecular form's (*a*) reduced clearance from the circulation [9], (*b*) high affinity to mesangial cell [6], and (*c*) enhancement of interleukin-6 expression, which induces immunoglobulin class switching to IgA [26, 27]. For these reasons, we next analyzed the molecular size of serum IgA in our model and found that serum macromolecular IgA levels in B6→HIGA mice were notably lower than in HIGA→HIGA mice (Fig. 6). Indeed, as reported in patients with AIDS, the increment of macromolecular IgA was not necessarily accompanied by mesangial IgA deposits [28, 29]. However, it is quite possible that the marked decrement of serum macromolecular IgA could attenuate mesangial IgA deposition in B6→HIGA mice because macromolecular IgA, which is a predominant constitute of the deposition [12], is decreased in the circulation. Our study may indicate that macromolecular IgA produced in mice with IgA nephropathy has a tendency to deposit into the mesangial area, unlike that of AIDS patients.

In addition to its role in IgA deposition, macromolecular IgA exerts its action variously in IgA nephropathy. Macromolecular IgA is a potent activator of complement [30], an inducer of matrix expansion [26, 31], and one of the molecules responsible for inducing proteinuria [32]. In this study, weaker mesangial C3 deposition, decreased glomerular sclerosis, milder matrix expansion, and less excretion of proteinuria were observed in B6→HIGA mice than the other experimental group, presumably because the B6→HIGA subjects had less macromolecular IgA.

Because BMCs transplanted into both B6→HIGA mice and HIGA→HIGA mice were equally exposed to the same environmental factors, the immunological differences in the two groups must come from variations in the BMCs. Accordingly, qualitative and quantitative changes of serum IgA may be determined at the level of stem cells. In support of this notion, we have obtained a result that BMT from IgA nephropathy-prone ddY mice to normal B6 mice increased the recipients' serum macromolecular IgA and glomerular IgA deposition [33]. We do not deny the involvement of antigens in the pathogenesis of IgA nephropathy. However, we consider that the antigens may merely elicit the basic factor that lies in BMCs. Still obscure is what factor(s) of BMCs, not of the environment, reduces macromolecular IgA in B6→HIGA mice. We speculate that bone marrow-derived cells, such as T cells [7, 34, 35], B cells [36, 37], Kupffer cells [9, 38–40] and mucosal intraepithelial cells [5, 41, 42], may contribute to determine qualitative and quantitative changes of serum IgA in HIGA mice and that the defects of these cells might be determined at the stem cell level. It was also thought that BMT from normal donors improved the abnormalities of bone marrow-derived cells and attenuated the glomerular lesions in these mice. We are now attempting to clarify which of these cells and what factors are defective at the stem cell level and are most important for increasing serum levels of macromolecular IgA in IgA nephropathy.

This study revealed that qualitative and quantitative changes in serum IgA are determined at the level of stem cells, and that disordered BMCs in mice with IgA nephropathy are responsible for high serum levels of IgA, especially macromolecular IgA, enough to cause glomerular lesions. Furthermore, our data implicate that BMT is therapeutically beneficial for murine IgA nephropathy and warrants a pioneering approach to study the pathogenesis of IgA nephropathy.

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APPENDIX

Abbreviations used in this article are: B6 mice, C57BL/6j mice; BMCs, bone marrow stem cells; BMT, bone marrow transplantation; ELISA, enzyme-linked immunosorbent assay; ESRD, end-stage renal

disease; FI, fluorescent intensity; FITC, fluorescein isothiocyanate; FPLC, fast protein, peptide, and polynucleotide liquid chromatography; HIGA mice, high IgA ddY mice; PAS, periodic acid-Schiff; PBS, phosphate-buffered saline; SRID, single radial immunodiffusion method.

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